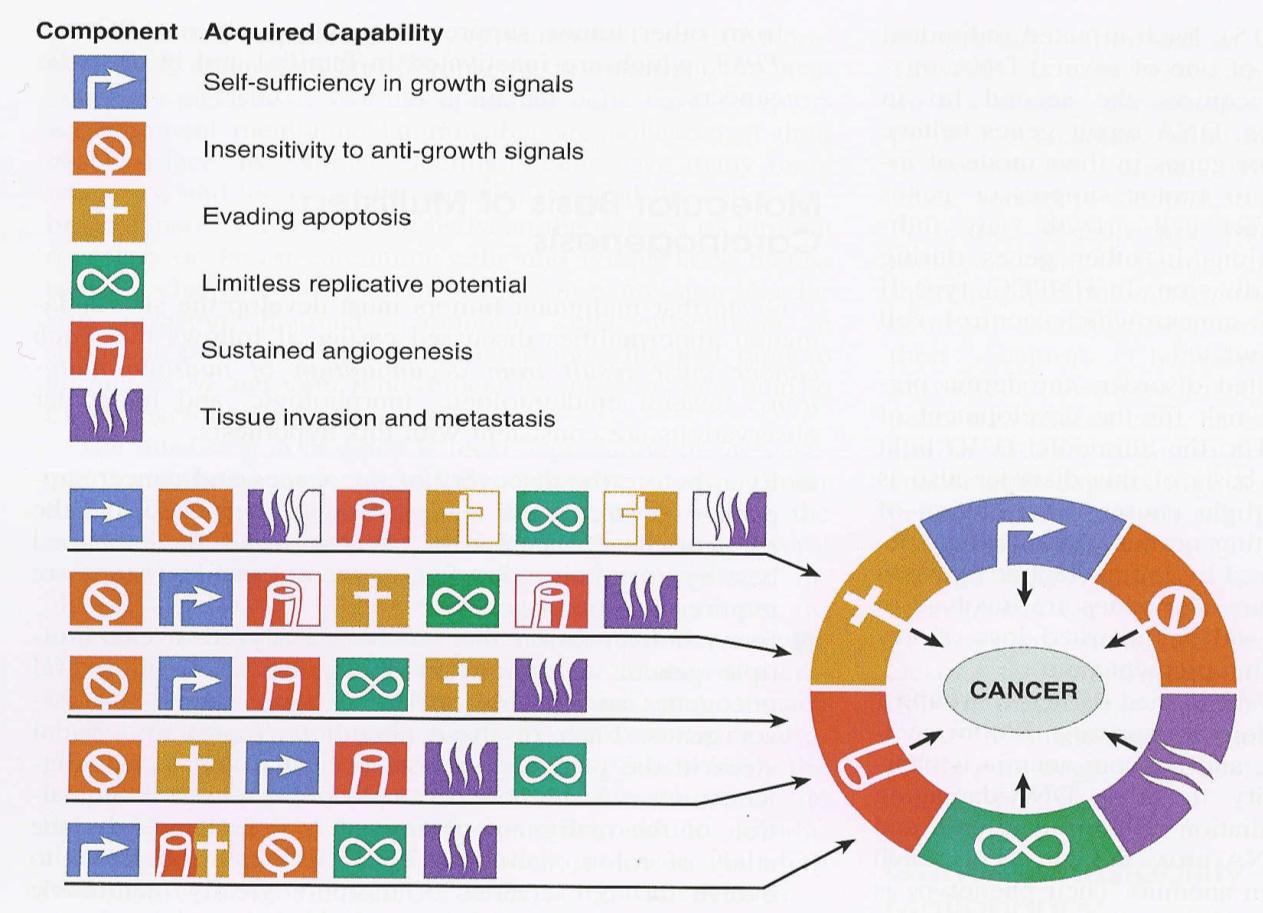
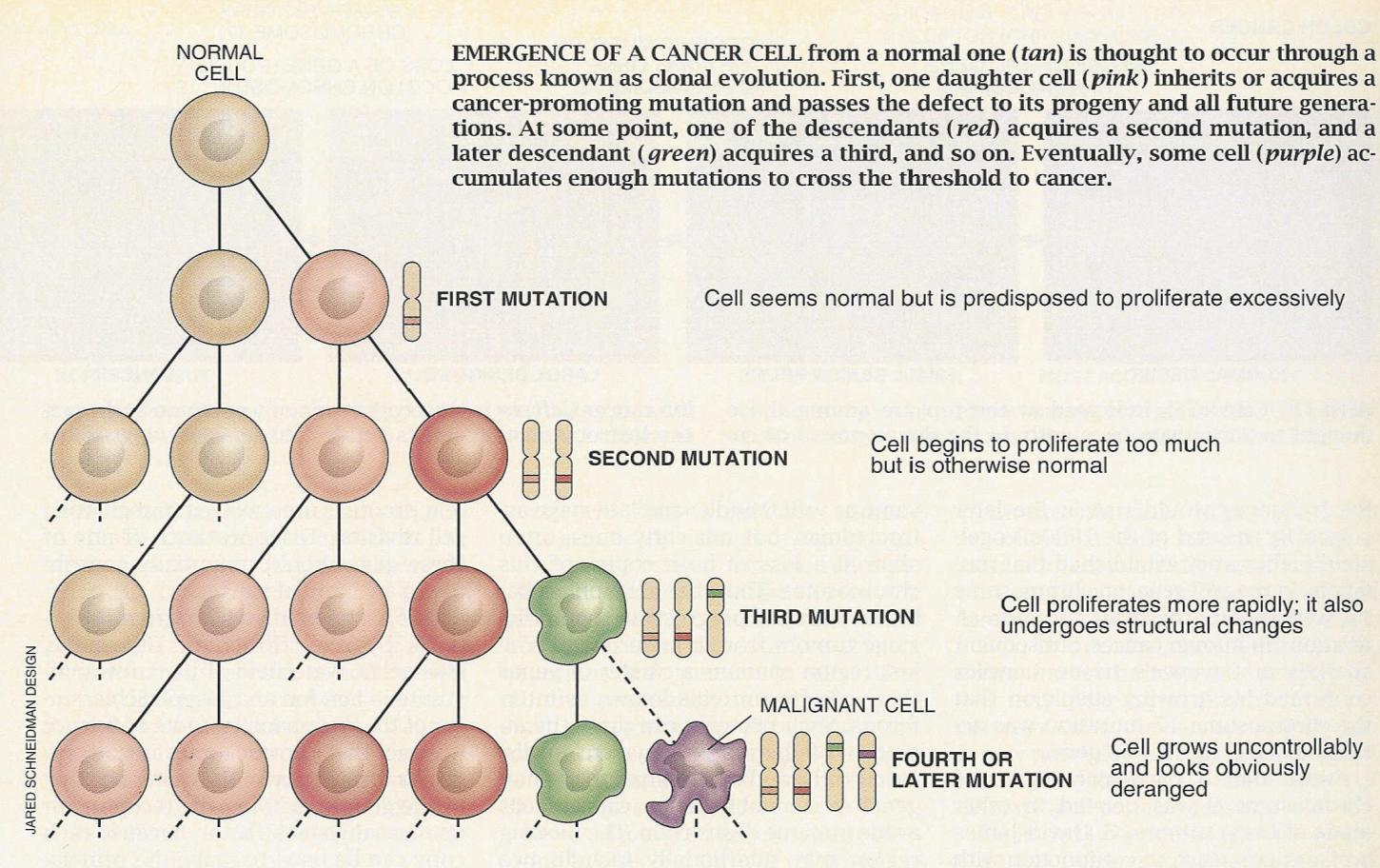
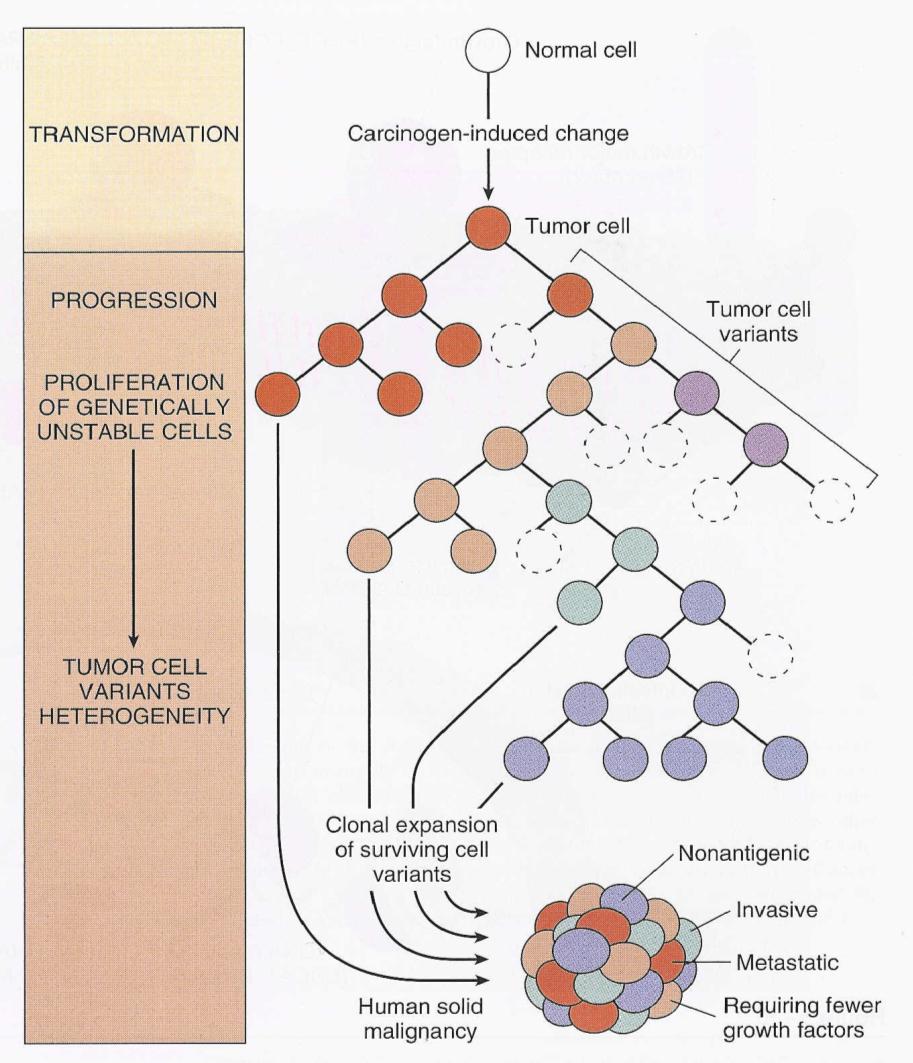
**Molecular Basis of Cancer**

* **Describe the principles of the molecular basis of cancer with respect to clonal development, cancer genes, their functions, their cellular localizations, and their control of the cell cycle**
  + **Molecular Basis of Cancer Development** 
    - Nonlethal genetic damage lies at the heart of carcinogenesis. May be acquired or inherited.
    - Tumor mass arises from the clonal expansion of a single progenitor cell that has incurred genetic changes (tumors are monoclonal).
    - Cancer results from multiple successive mutations in genes that control cell growth, differentiation, apoptosis or that maintain genomic integrity.
    - Though malignant tumors are monoclonal in origin, the constituent cells are heterogeneous, due to additional genetic alterations that create “subclones”.
    - These cells posses a growth advantage over their normal counterparts.
  + **Multiple Pathways of Carcinogenesis**
    - ****
      * not a set plan, doesn't need to follow a specific order
  + **Emergence of a Cancer Cell**
    - ****
  + **Tumor Progression and Generation of Heterogeneity**
    - This panel illustrates clonal evolution of tumors and generation of tumor cell heterogeneity. New subclones arise from the descendants of the original transformed cell, and with progressive growth the tumor mass becomes enriched for those variants that are more adept at evading host defenses and are likely to be more aggressive.
      * cells are genetically unstable, prone to further mutation leads to clonal variance
    - ****
  + **Categories of Genes Responsible for Tumorigenesis**

**Gene Mechanism of Transformation Examples**

(Proto)oncogene Gain of function myc,myb

Signal transduction molecules bcr/abl

and transcription factors

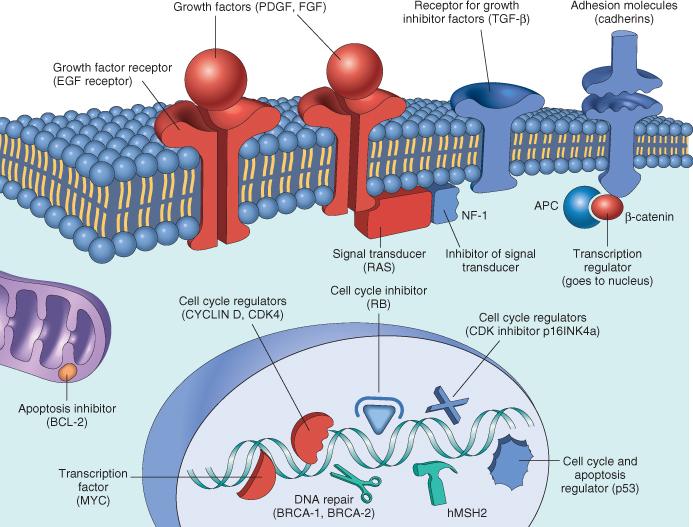
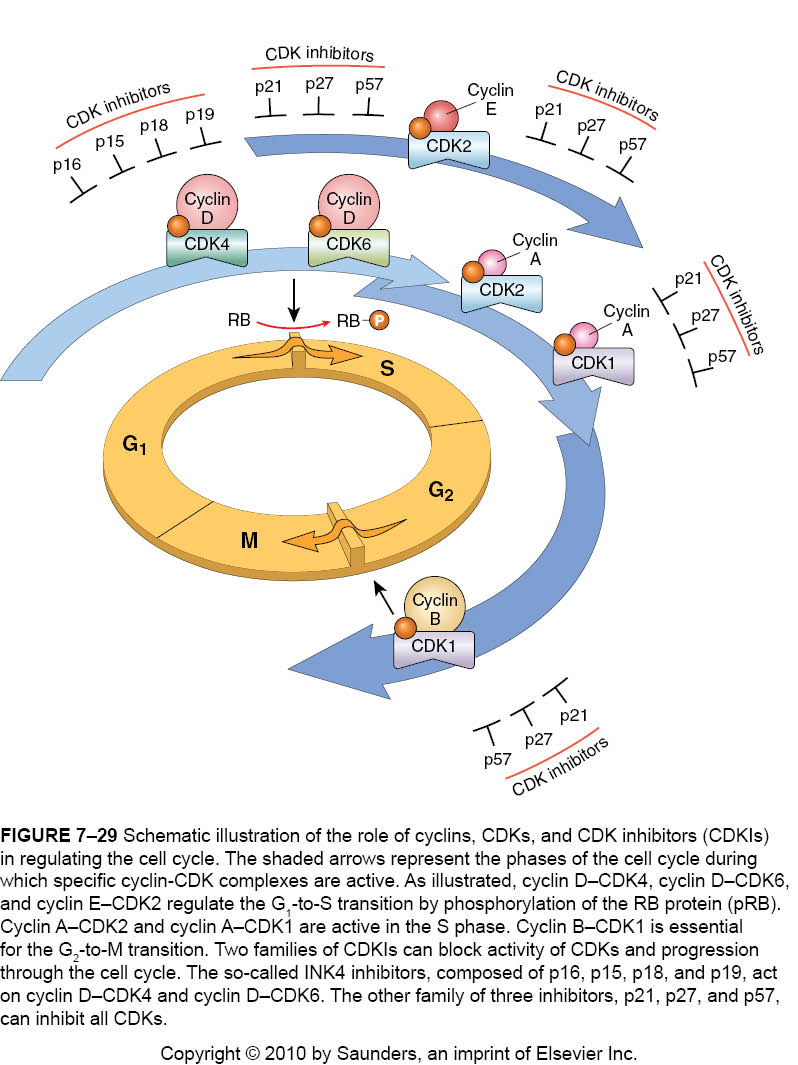
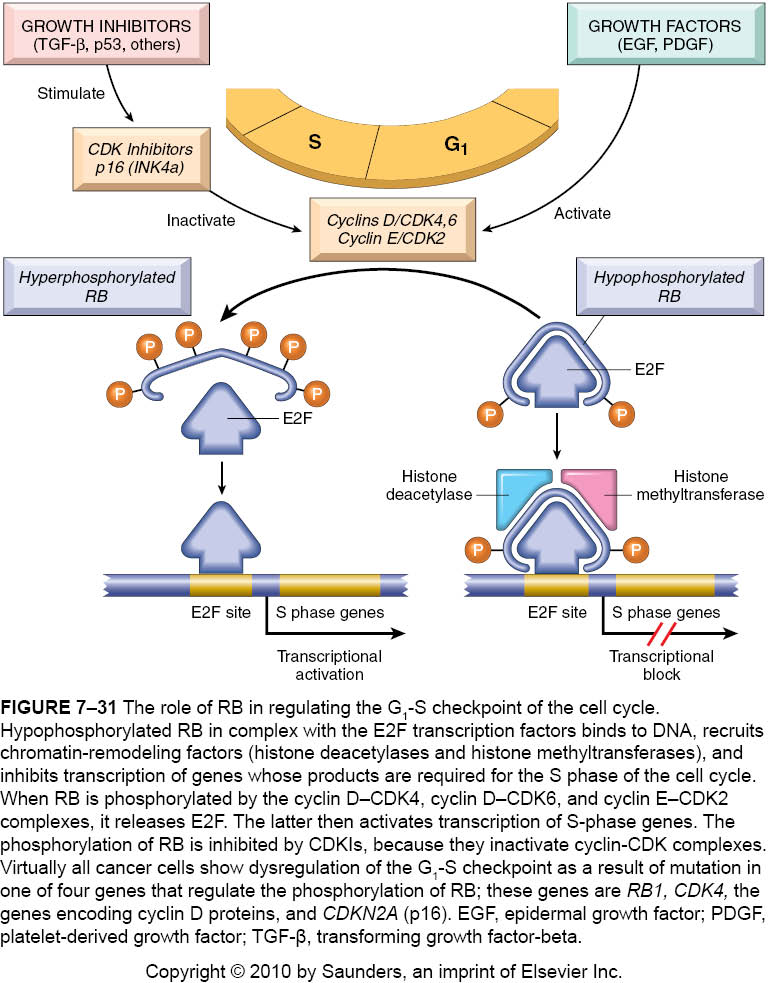
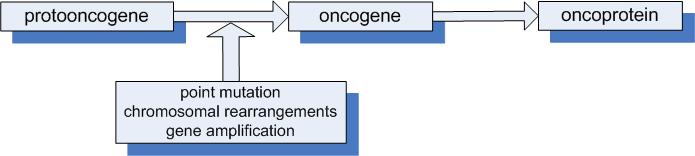
Tumor Suppressor Gene Loss of function Rb, p53,

Control of cell cycle BRCA-1,2

Apoptosis Gene Disruption of programmed bcl-2

cell death

DNA Repair Gene Disruption of normal DNA repair msh-2,mlh-1

* + **Subcellular Localization and Functions of Cancer Genes**
    - ****
  + **The Normal Cell Cycle**
    - Non-dividing cells are in the G0 stage and need to be recruiting into the G1 stage and beyond in order to undergo replication.
    - The orderly progression of cells through the various phases of the cell cycle is orchestrated by cyclins and cyclin-dependent kinases (CDKs) and their inhibitors.
    - Phosphorylation of RB (retinoblastoma susceptibility protein) is a molecular on-off switch for cell cycle.
    - Phosphorylation of RB eliminates the barrier to cell cycle progression and promotes replication.In quiescent cells, hypophosphorylated RB binds to a protein complex that includes E2F & DP1.
    - E2F/DP1/RB binds to promotors of E2F-responsive genes causing them to be silent.
    - Stimulation by growth factors → ↑ cyclins D and E → cyclin D-CDK4 and cyclin E-CDK2 complexes → phosphorylation of RB → active E2F → transcription of genes essential for progression through S phase
  + **Regulation of the Cell Cycle**
    - 
  + **Cell Cycle Regulation by the *Rb* Gene**
    - 
  + **Oncogenes**
    - Protooncogenes - promote normal growth and differentiation (code for proteins that trigger cell division/cell cycle progression)
    - Protooncogenes can be converted into oncogenes by genetic alterations.
    - Oncoproteins are devoid of regulatory elements. Their production in the transformed cell does not depend on growth factors or other external signals.
    - Dominant acting/gain of function
    - 
    - **Protein Products of Oncogenes**
      * Increased production of secreted growth factor
        + PDGF, FGF
      * Increased expression of growth factor receptors
        + EGFR
      * Mutant signal transducer proteins
        + *ras, abl*
      * Mutant transcription factor production
        + *myc*
      * Overproduction of factor that prevents apoptosis
        + *bcl-2*
      * Abnormal expression of cell cycle regulator proteins
        + Cyclin D, CDK
    - **Mechanisms of Protooncogene Activation**
      * Quantitative (Increase in amount of oncogene product or production in inappropriate cell types) -- normal protein in abnormal amounts
        + Insertion

Insertion of retroviral genome

Chromosome translocation

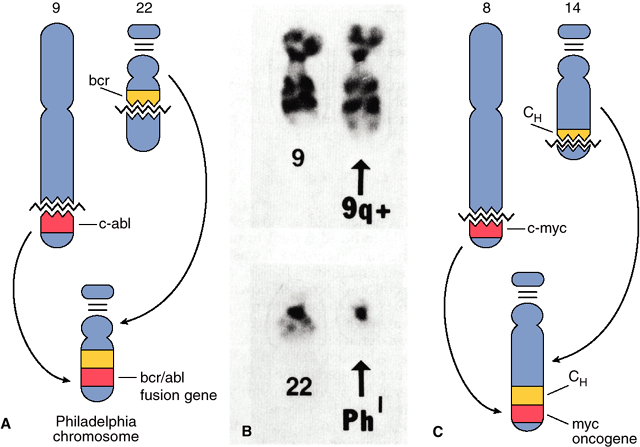
* + - * + Gene amplification

erbB2 (Her2/Neu) and c-myc amplification in 40% of breast cancers

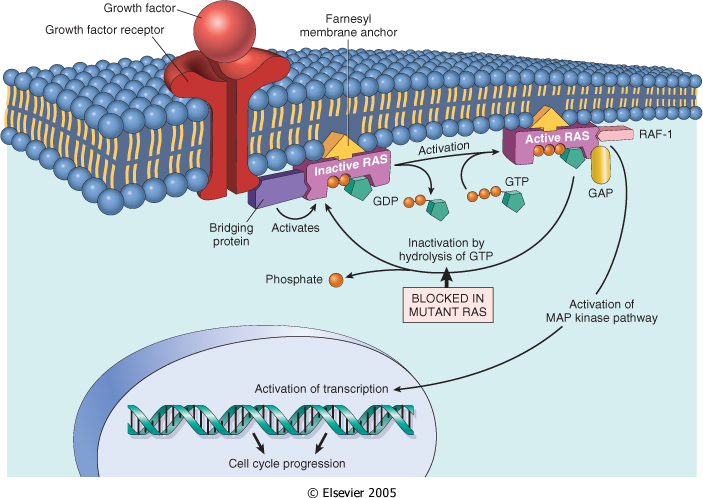
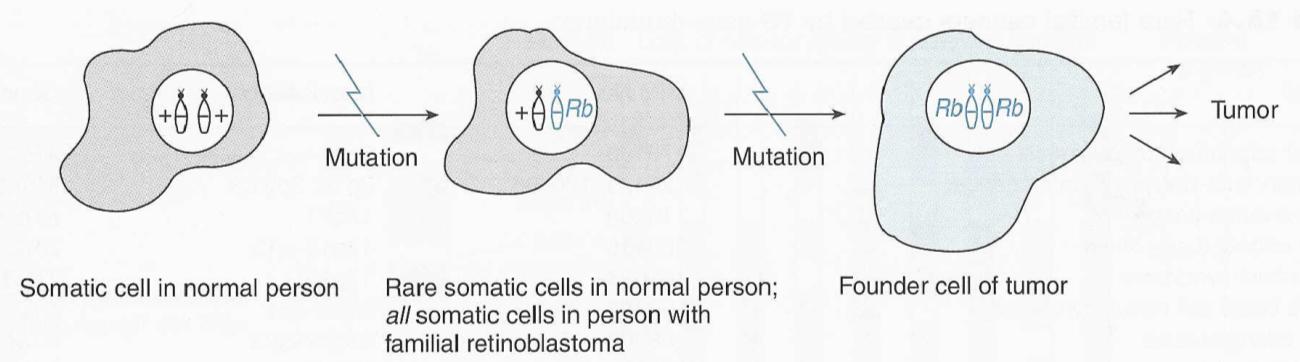
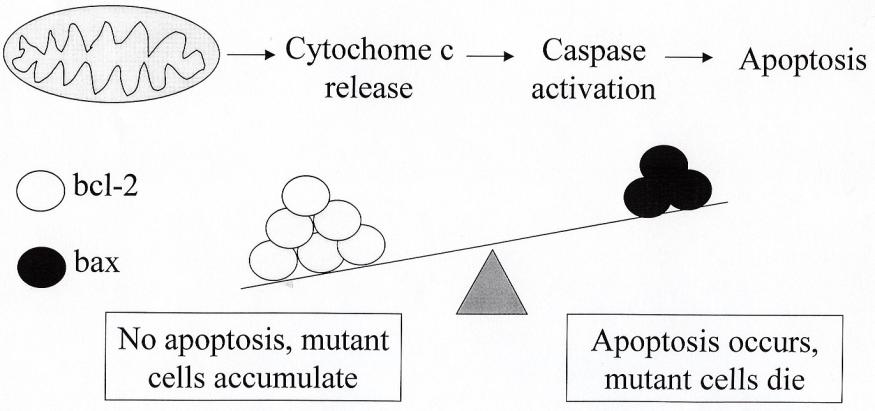
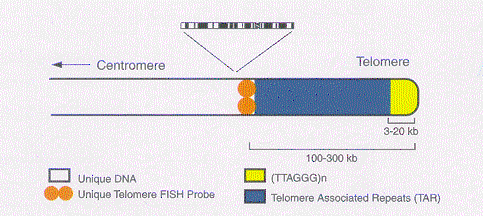
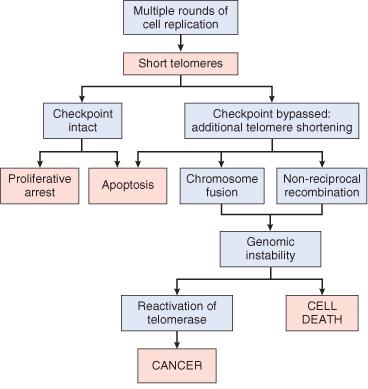
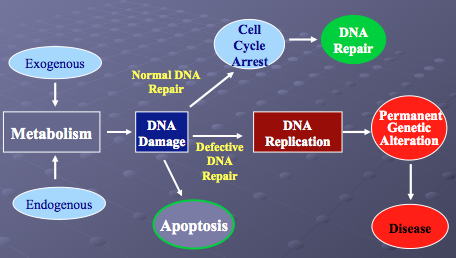
N-myc amplification in neuroblastoma with poor prognosis

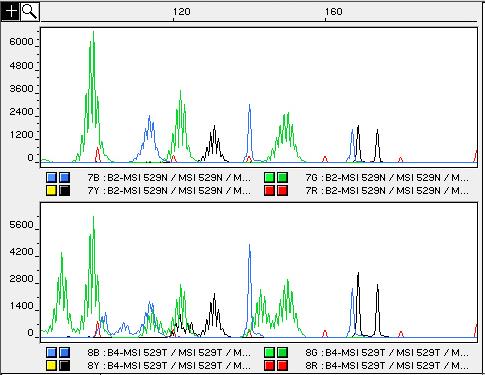
* + - * Qualitative (Alteration of nucleotide sequence confers new properties)-- normal amnts, but new seq w/ new properties and will not respond to growth inhib
        + Point mutation
        + Chromosome translocation
    - **Example of Oncogene Activation by Chromosomal Translocation**

CML Burkitt Lymphoma

* + - * 

affects transcription Ig heavy chain next to myc oncogene

* + - ***RAS* Oncogene**
      * Best example of signal transducing protein.
      * Point mutations of *RAS* family genes is the most common abnormality of dominant oncogenes in human cancers.
      * Inactive, *RAS* proteins bind GDP; when stimulated by growth factors *RAS* activated → exchange GDP for GTP → signal transmission.
      * Active state is normally transient since intrinsic GTPase activity hydrolyzes GTP to GDP → inactive *RAS*.
      * Mutant *RAS* proteins bind GAP *(GTPase-activating proteins)* but their GTPase activity fails to be augmented
      * 
      * signal constantly on bc constantly constitutively bound to GTP
  + **Tumor Suppressor Genes**
    - Code for proteins that down-regulate cell division
      * Recessive acting/loss of function
      * Expression inhibits cancer formation
    - Knudson’s two-hit hypothesis of cancer development: Both alleles are inactivated by mutation or deletion.
      * in some cases first inact copy can be inherited from parent (or can just happen)
    - **Tumor Suppressor Genes or Their Protein Products are Inactivated in Cancer**
      * One gene copy is mutated, while the other is lost (allelic deletion or loss of heterozygosity).
        + Most mutations are acquired (somatic)
        + Some are inherited (Knudson’s hypothesis)
        + First hit (mutation) is inherited; second hit results from somatic mutation or loss (chromosomal deletion)
      * 
    - **Retinoblastoma**
      * *Rb* gene is the prototype of the two-hit hypothesis
      * Childhood retinal tumor
      * 40% cases are familial, 60% are sporadic
      * Both normal alleles of the *Rb* locus must be inactivated for the development of retinoblastoma.
        + In familial cases, children inherit one defective copy of the *Rb* gene in the germ line; the other copy is normal. Retinoblastoma develops when the normal *Rb* gene is lost in the retinoblasts as a result of somatic mutation.
        + In sporadic cases, both normal *Rb* alleles are lost by somatic mutation in one of the retinoblasts.
      * Cancer develops when the cell becomes homozygous for the mutant allele
      * Patients with familial retinoblastoma are at risk for developing other tumors
    - **Other Tumor Suppressor Genes**
      * **p53 gene**: mutated in >50% of human cancers
        + Li-Fraumeni syndrome - inherit a mutant copy of p53 gene - 25X increased risk of many cancers.
      * ***BRCA-1 and BRCA-2:*** function in DNA repair
        + Mutations account for 80% of familial breast cancers.
        + BRCA-1 - breast and ovarian
        + BRCA-2 - male breast, ovary and possibly others
      * ***APC* gene:** “Adenomatous polyposis coli”
        + APC gene (5q21) codes a protein which sequesters β-catenin and prevents it from activating myc transcription
        + Patients inherit one mutant allele, develop hundreds or thousands of polyps and inevitably develop colon cancer in adulthood.
* **Describe the roles of apoptosis and telomerase activation in cancer development**
  + **Genes that Regulate Apoptosis**
    - Prototype **= *bcl-2***
      * Prevents apoptosis by sequestering cytochrome c in the mitochondrion
      * Other members of *bcl-2* family promote apoptosis (eg. *bax*) by allowing its release.
      * The ratio of death antagonists and agonists determines whether a cell will respond to an apoptotic stimulus.
      * Overexpression of bcl-2 extends cell survival → more mutations → malignancy (example: B-cell follicular lymphomas🡪 overexpression of BCL2 allows for the cell to stick around longer and more mutations to occur)
      * 
  + **Human Chromosome Telomere** 
    - At birth human telomeres are approximately 15,000 bp of (TTAGGG)n and this sequence shortens with each cell division by 25–200 bp.
    - After approximately 100 cycles of reduction (shortening at each cell division), cells senesce (age) and can no longer divide.
    - Telomerase activity protects and stabilizes (maintains) the integrity of chromosomal telomeres. (Present in germ cells.)
    - In most somatic cells, telomerase is absent and hence they suffer progressive loss of telomeres.
    - 
    - **Cellular Responses to Telomere Shortening**
      * Telomerase activity is expressed in many human tumors. Maintenance of telomere length inhibits cell senescence, resulting in cells with a distinct growth advantage and increased risk of acquiring new genetic alterations.
      * 
* **Describe the role of DNA damage and defective repair in cancer development and genetic disease**
  + **Genes that Regulate DNA Repair**
    - 
    - Act indirectly by correcting errors in DNA that occur spontaneously during cell division or that occur during exposure to mutagenic chemicals or irradiation.
    - Those born with inherited mutations of DNA repair proteins are at a greatly increased risk of developing cancer because the mutated genes allow mutations in other genes during the process of normal cell division.
    - Examples:
      * Xeroderma pigmentosum
        + NER
      * Ataxia telangiectasia
        + ATM
      * HNPCC (hereditary nonpolyposis colon cancer)
  + **Xeroderma Pigmentosum**
    - Defective nucleotide excision repair (NER pathway)
    - Skin pigmentation
    - Neurologic abnormalities
    - Autosomal recessive
    - Increased risk for developing cancers of the skin following exposure to UV light in sun rays.
      * squamous, basal cells, potentially melanoma
  + **HNPCC--Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)**
    - believed dz is underrecognized. causes endometrial probs as well.
    - Familial carcinomas of the colon affecting predominantly the cecum and proximal colon.
    - Defects in genes involved in DNA mismatch repair.
    - One of the hallmarks of patients with mismatch-repair defects is microsatellite instability (MSI).
      * Microsatellites are short, repeated DNA sequences composed of repeat units that are 1 to 6 base pairs in length.
      * Repeats are distributed throughout the human genome and often vary in length from one individual to another
      * MSI is an acquired change in length of a microsatellite resulting from insertion or deletion of repeat units during DNA replication, and concomitant failure of the DNA mismatch repair system to correct these errors.
  + **Molecular Diagnostic Application:** Detection of defective, mutated mismatch repair genes (hMSH1, hMLH1) by assessment of microsatellite instability
    - Detected in 90% of Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC, or Lynch Syndrome), and 10-15% of sporadic colorectal carcinomas
    - MSI testing:
      * A fluorescent PCR (polymerase chain reaction)-based assay
      * Microsatellite regions are amplified and detected by capillary electrophoresis
      * Compares allelic profiles of microsatellite markers generated by amplification of DNA from matching normal and tumor tissues.
      * The presence of alleles in the tumor sample that are not found in the normal sample from the same individual indicates MSI.
        + key is finding alleles not in norm sample



NR-21

BAT-26

BAT-25

NR-24

MON0-27

Penta C

Penta D

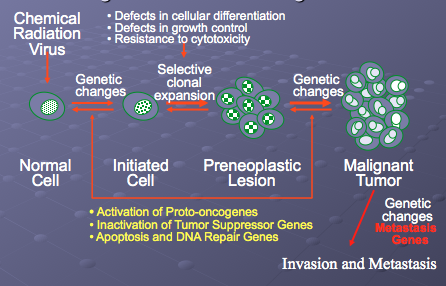
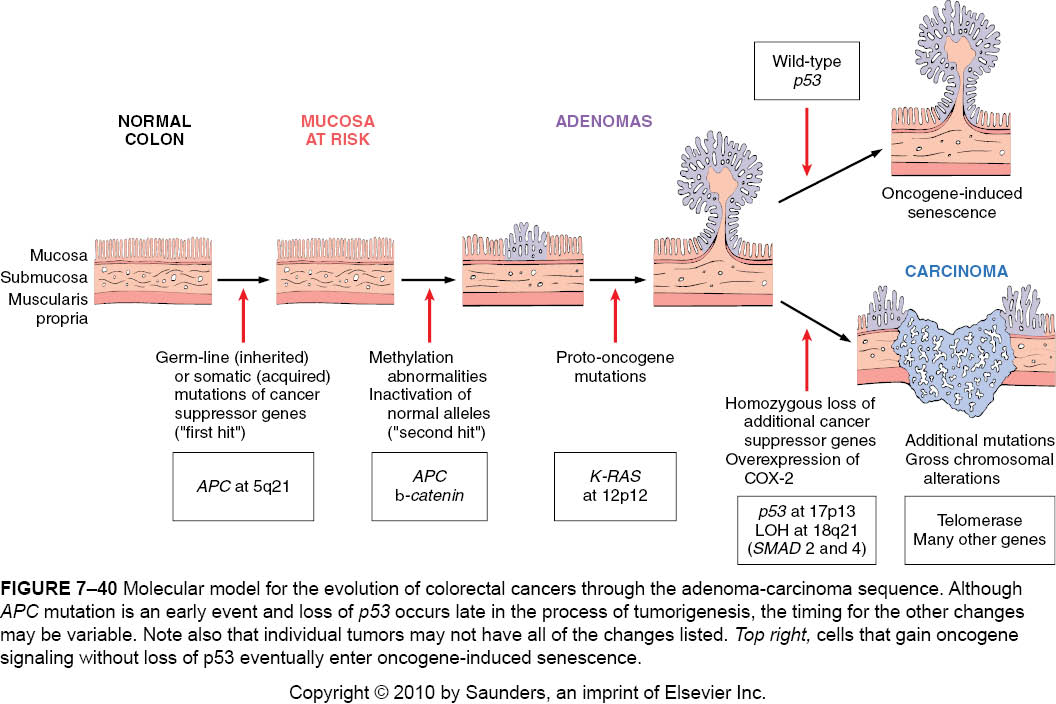
Penta D

Penta C

* + - * + ^^ see additional seq that aren't normally there
        + 28 yr old male with colon cancer; family history of early colon cancer (mother 32 years, brother 20 years).
        + Microsatillite Instability Assay Results:

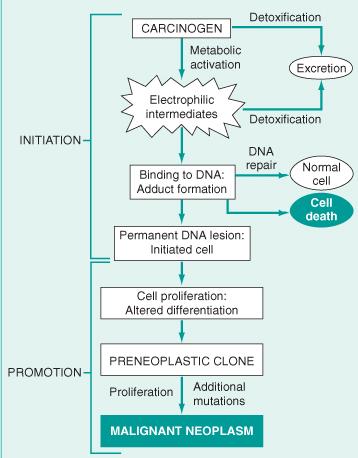
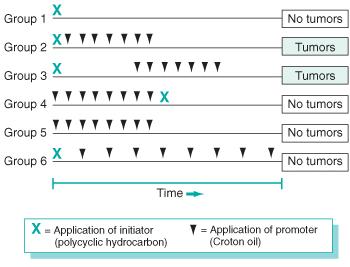
shift at 5/5 markers in the patient’s tumor sample demonstrating high microsatellite instability

The name of each microsatellite is shown above the appropriate peak.

* **Describe the concept of the “multi-step nature of carcinogenesis”**
  + **Carcinogenesis Is a Multistage Process**
    - ****
  + **The Molecular Pathogenesis of Human Colorectal Cancer**
    - ****
  + **Molecular Basis of Multistep Carcinogenesis**
    - In vitro (and in vivo), no single genetic alteration can induce malignant transformation.
    - Multiple controls, (oncogenes, tumor-suppressor genes and apoptosis regulating genes) must be lost for the emergence of cancer cells.
    - Most human cancers that have been analyzed reveal multiple genetic alterations involving activation of several oncogenes and loss of two or more tumor suppressor genes.
    - All cancer is therefore genetic, but only a small subset is hereditary.
* **Describe the concepts of chemical, microbial and radiation carcinogenesis as well as selected agents for each**
  + **Carcinogenic Agents**
    - **Chemicals**
      * **Initiation**
        + **Results from exposure of cells to sufficient dose of a carcinogenic agent (*initiator*).**
        + **Causes irreversible DNA damage**
        + **Two categories:**

**Direct acting - do not require chemical transformation**

**Indirect-acting (procarcinogens) - require metabolic conversion in vivo to produce ultimate carcinogens capable of transforming cells**

* + - * **Promotion**
        + **Tumor induction in previously initiated cells (agent = *promoter*)**
        + **Promoters are nontumorigenic by themselves**
        + **Cellular changes do not affect DNA directly and are reversible**
      * ****
        + grp 6 longer interval maybe cells senesced. anyway no cancer
      * **Alkylating agents** - cyclophosphamide, bulsulfan (direct-acting) used in Rx cancer (↑ risk another neoplasm)
        + actually used for cancer therapy
      * **Aromatic hydrocarbons** - cigarette smoke
      * **Azo dyes** - β-naphthylamine (aniline dye) bladder cancer (used in production of polyurethane)
        + occupational exposure
      * Naturally occurring carcinogens - **Aflatoxin B**1 produced by *Aspergillus* *flavus* - associated with HCC in Africa
      * **Nitrosamines** and **amides** - can be synthesized in GI tract from ingested nitrites and contribute to gastric cancer.
      * Miscellaneous agents - **Asbestos, vinyl chloride, nickel**
        + Asbestos-- mesothelioma
    - **Radiant energy**
      * **Ultraviolet Rays**
        + Increased incidence of squamous cell carcinoma, basal cell carcinoma and possibly malignant melanoma of the skin.
        + Risk depends on type, intensity and quantity of melanin in the skin (↑ fair skin & lots of sun i.e. Australia, New Zealand)
        + UVB (280-320 nm) most potent mutagen, by forming pyrimidine dimers
        + Repaired by nucleotide excision repair (NER) pathway;

Xeroderma pigmentosum - autosomal recessive - inability to repair UV induced DNA damage - 2000X increased risk in sun-exposed skin.

* + - * **Ionizing Radiation**
        + Overwhelming evidence for the carcinogenicity of electromagnetic (x-rays, γ-rays) and particulate radiations (α-particles, β-particles, protons, neutrons).

Miners of radioactive elements in Europe and U.S. have 10X increase in lung cancer

Survivors of atomic bomb explosions (Hiroshima, Nagasaki)

Therapeutic radiation (thyroid ca s/p head & neck radiation)

Inhabitants of Marshall Islands were H-bombs were tested

Increase in thyroid cancer in areas near Chernobyl power plant accident

proven increased thyroid carcinoma

* + - * + Organ sensitivity to ionizing radiation

High - myelopoietic tissue, thyroid; Moderate - breast, lungs, salivary gland; Low - skin, bone, GI tract

* + - **Oncogenic viruses and other agents**
      * **DNA viruses**
        + Transforming DNA viruses integrate into and form stable associations with the host cell genome.
        + Viral genes that are transcribed early (early genes) are important for transformation and are expressed in transformed cells.
        + Examples

**Human Papilloma Virus (HPV)**

>70 genetically distinct types

Implicated in:

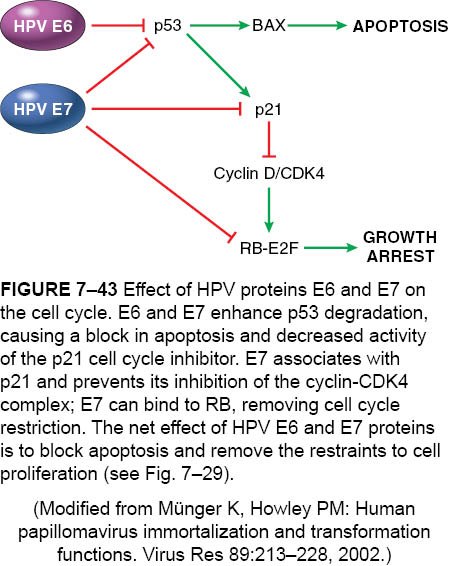
squamous cell carcinoma of the cervix and anogenital region

oral and laryngeal cancers

DNA sequences of HPV 16 and 18 are present in 85% of invasive squamous cell cancers, cervical dysplasia and carcinoma in-situ (“high-risk”)

HPV 6 and 11 are associated with genital warts (“low-risk”)

E6 and E7 proteins of high-risk HPV disable important tumor suppressor proteins that regulate the cell cycle, p53, p21 and RB.



**Epstein-Barr Virus (EBV)**

Member of the Herpes family

Implicated in:

African form of Burkitt lymphoma

B-cell lymphomas in immunosuppressed patients

Some cases of Hodgkin lymphoma

Nasopharyngeal carcinomas

EBV infects B-cells (and possibly epithelial cells of the oropharynx).

Uses complement receptor CD21 to attach and infect B-cells.

Infection of B-cells is latent; cells are immortalized.

**EBV: Burkitt Lymphoma**

Neoplasm of B-lymphocytes

Most common childhood tumor in Central Africa and New Guinea

Association with EBV is strong:

>90% of African tumors carry the EBV genome

100% of patients have antibody to viral capsid antigens

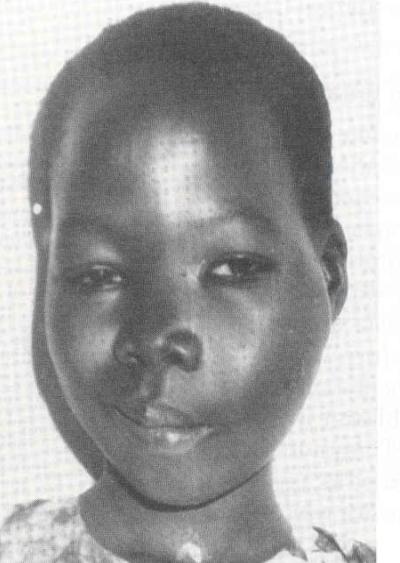
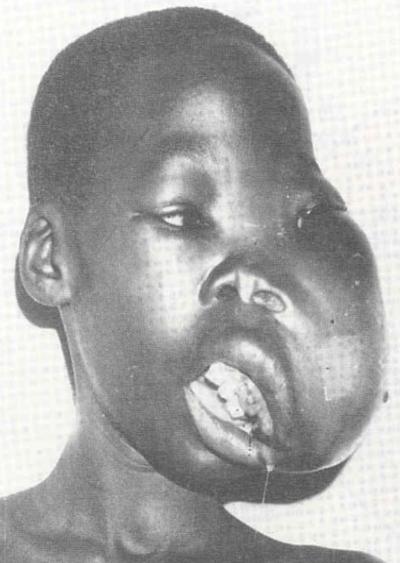
Serum antibody titers against viral capsid antigens are correlated with the risk of developing the tumor

Other factors in addition to EBV must be involved:

EBV is ubiquitous (not just seen where Burkitt lymphoma is found)

EBV causes infectious mononucleosis (self-limited)

EBV is found in only 15-20% of Burkitt lymphoma outside Africa; but all tumors have t(8,14)



Before Tx 3.5 wks after tx w/ cyclophoosphamide

**B-cell Lymphoma**

Immunosuppressed patients:

AIDS

Patients on long-term immunosuppressive therapy for preventing allograft rejection

posttransplant lymphoproliferative problems

Tumors are polyclonal at outset but can develop into monoclonal neoplasms

In some cases the tumors regress after relaxation of immunosuppressive therapy

Nasopharyngeal Carcinoma

Endemic in Southern China, some parts of Africa, and the Inuit population of the Arctic

100% of these tumors contain EBV DNA

The restricted geographic distribution indicates that genetic or environmental factors, or both, contribute to tumor development

**Hepatitis B Virus (HBV)**

70-85% of hepatocellular carcinomas worldwide are associated with Hepatitis B or C.

mechanism diff than other DNA virus

HBV is endemic in countries of the Far East and Africa; consequently they also have the highest incidence of HCC

In Taiwan those infected have 200X greater risk of HCC compared with uninfected in the same area

Role is not clear. HBV and HCV genomes do not encode any viral oncoproteins. HBV DNA does integrate with human genome, but there is no consistent pattern.

theories:

Immunologically mediated chronic inflammation with hepatocyte death leading to regeneration and genomic damage.—hepatocytes are more susceptible

Activation of NF-B pathway in hepatocytes, in response to activated immune cells, blocks apoptosis.

HBV encodes *HBx protein* that can directly or indirectly activate a variety of transcription factors and signal transduction pathways.

* + - * RNA viruses
        + T-Cell Leukemia Virus Type I (HTLV-1)

Only retrovirus firmly implicated in the causation of cancer.

Endemic in certain parts of Japan and the Caribbean basin (sporadically elsewhere)

Has tropism for CD4+ T-cells (like HIV)

Infection requires transmission of infected T-cells via sexual intercourse, blood products or breast feeding

Leukemia develops in 3-5% of infected individuals after a latent period of 40-60 years

In addition to leukemia, HTLV-1 is associated with a demyelinating neurologic disorder (tropical spastic paraparesis)

Molecular mechanism not clear, but the virus gene *tax* causes proliferation of T-cells.

* + - * Others
        + Helicobacter pylori

Gastric infection linked with gastric lymphomas and carcinomas

H. pylori infection detected in the majority of gastric lymphomas

Treatment of the infection with antibiotics results in regression of the lymphoma in most cases

Tumors arise in mucosa-associated lymphoid tissue (called **MALTomas**); also the B-cells that give rise to these tumors reside in marginal zone of lymphoid follicles, AKA marginal zone lymphoma)

Infection initially generates *H. pylori*-reactive T-cells, which activate a polyclonal population of B-cells; B-cells eventually become monoclonal and independent

so at UT look for H pylori in all gastric biopsies just in case. do special stain

SUMMARY

Cancer is a genetic disease that:

* Is clonal in nature
* Is characterized by mutations in multiple classes of genes that result in:
  + Uncontrolled cell growth and proliferation
  + Failed tumor suppression
  + Failed apoptosis
  + Failure to repair DNA damage
* Is characterized by a multistep process whereby multiple mutations are required to bring about malignancy
* Can be characterized by specific cytogenetic and molecular markers
* Is caused by exposure to various carcinogenic agents that have specific molecular consequences.